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Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones

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Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones

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Abstract

Low energy availability, defined as low caloric intake relative to exercise energy expenditure, has been linked to endocrine alterations frequently observed in chronically energy-deficient exercising women. Our goal was to determine the endocrine effects of low energy availability in exercising men. Six exercising men (V_{O_2peak}: 49.3 ± 2.4 ml · kg^{-1} · min^{-1}) underwent two conditions of low energy availability (15 kcal · kg^{-1} fat-free mass [FFM] · day^{-1}) and two energy-balanced conditions (40 kcal · kg^{-1} FFM · day^{-1}) in randomized order. During one low energy availability and one balanced condition, participants exercised to expend 15 kcal · kg^{-1} FFM · day^{-1}; no exercise was conducted during the other two conditions. Metabolic hormones were assessed before and after each 4-day period. Following both low energy availability conditions, leptin (−53% to −56%) and insulin (−34% to −38%) were reduced ($P < 0.05$). Reductions in leptin and insulin were independent of whether low energy availability was attained with or without exercise ($P > 0.80$). Low energy availability did not significantly impact ghrelin, triiodothyronine, testosterone and IGF-1 (all $P > 0.05$). The observed reductions in leptin and insulin were in the same magnitude as changes previously reported in sedentary women. Further research is needed to understand why other metabolic hormones are more robust against low energy availability in exercising men than those in sedentary and exercising women.

Keywords: Energy deficiency, caloric restriction, exercise, leptin, testosterone

Introduction

It is generally recommended that people who regularly engage in exercise increase their dietary energy intake to match the additional energy requirements of exercise (Rodriguez, Di Marco, & Langley, 2009). However, energy balance is not always the objective of exercise, and it is well established that many exercisers fail to meet their energy requirements (Loucks, 2004; Loucks, Kiens, & Wright, 2011). Reasons for chronically or excessively low energy intakes in exercising populations include misguided attempts to lose or maintain a low body weight, disordered eating, clinical eating disorders and inadvertent undereating (Nattiv et al., 2007).

Even though an energy deficit is required for successful weight loss and the prevention and treatment of overweight, obesity and associated comorbidities (Donnelly et al., 2009), chronic or excessive energy deficits can be associated with negative outcomes, particularly in non-overweight populations. Chronic energy deficiency can result in the suppression of dispensable metabolic processes, such as growth and reproduction, to conserve energy for vital functions (Wade, Schneider, & Li, 1996). These metabolic alterations are associated with unfavorable health outcomes, such as impaired reproductive, bone and cardiovascular health (De Souza et al., 2007, 2008; De Souza & Williams, 2004, 2005; Ducher et al., 2011; O'Donnell, Harvey, & De Souza, 2009). These health effects have been reported primarily in exercising women and have been captured under the term *female athlete triad* (De Souza et al., 2014; Nattiv et al., 2007). However, observational studies suggest that male athletes, particularly those competing in sports that emphasize leanness or a low body weight, demonstrate an increased propensity for disordered eating (Martinsen, Bratland-Sanda, Eriksson, & Sundgot-Borgen, 2010; Rosendahl, Bormann, Aschenbrenner, Aschenbrenner, & Strauss, 2009) and that reproductive function and bone health may also be impaired in exercising men at risk of chronic energy deficiency (Campion et al., 2010; De Souza, Arce, Pescatello, Scherzer, & Luciano, 1994; Dolan et al., 2012; Hagmar, Berglund, Brismar, & Hirschberg, 2013).

Seminal studies by Loucks et al. have demonstrated that the underlying metabolic alterations are directly linked to low energy availability, rather than to exercise per se (Hilton & Loucks, 2000; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks, Verdun,

& Heath, 1998). Energy availability is defined as the difference between dietary energy intake and the energy expended during exercise (Loucks, 2004), and can be understood as the amount of energy that remains for physiological processes after deducting the energy cost of exercise (Loucks et al., 2011). Thus, low energy availability can be attained through low caloric intake, high exercise energy expenditure or a combination of both. Well-controlled laboratory experiments have demonstrated that an energy availability below 30 kcal · kg^{-1} fat-free mass (FFM) · day^{-1} is associated with the suppression of key metabolic hormones, such as leptin, insulin, insulin-like growth factor 1 (IGF-1) and triiodothyronine (T3) (Hilton & Loucks, 2000; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks et al., 1998).

It is notable that controlled experiments on the metabolic effects of low energy availability have only been conducted in sedentary women (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks et al., 1998); no controlled experiments have utilized men, and particularly exercising men. Reductions in T3, IGF-1 and testosterone have been reported in field studies in male soldiers exposed to various levels of energy deficiency during military training (Friedl et al., 2000; Kyrolainen et al., 2008; Nindl et al., 2007), which suggests that key metabolic hormones may also be suppressed in severely energy-deficient exercising men. However, male reproduction requires considerably less energy than that of females (Bronson, 1985), and it is likely that the impact of low energy availability on endocrine function, and particularly reproductive function, is less pronounced in men. Therefore, controlled experiments are needed to characterize the relationship between low energy availability and hormonal and metabolic alterations in this population.

To this end, the purpose of the present study was two-fold: (1) to determine the impact of a controlled reduction in energy availability to 15 kcal · kg^{-1} FFM · day^{-1} on metabolic hormones (leptin, ghrelin, insulin, free T3, testosterone, IGF-1) and metabolic substrates (glucose, glycerol, free fatty acids (FFA)) in exercising men and (2) to explore whether the effects of low energy availability on these same outcomes differ when low energy availability is attained (a) through caloric restriction without exercise or (b) through caloric restriction in combination with exercise. We hypothesized that low energy availability would be associated with reductions in leptin, insulin, free T3, testosterone, IGF-1 and glucose and increases in ghrelin, glycerol and FFA. We further hypothesized that these alterations would not differ when compared between low energy availability attained through caloric restriction alone (a) or through a combination of caloric restriction and exercise (b).

Methods

Experimental design

The present study utilizes a repeated-measures cross-over design with four experimental conditions (Figure 1). Energy intake and exercise energy expenditure were manipulated such that each participant completed two conditions of low energy availability (15 kcal · kg^{-1} FFM · day^{-1}) and two conditions of normal energy availability (40 kcal · kg^{-1} FFM · day^{-1}), operationally

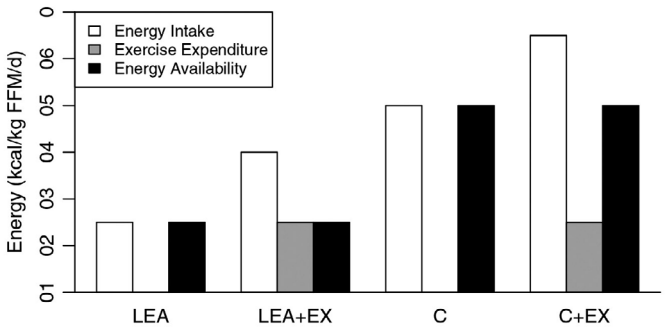

Figure 1. Schematic display of the study design.

defined as control conditions. Participants expended 15 kcal · kg^{-1} FFM · day^{-1} during supervised exercise during one low energy availability condition (LEA + EX) and one control condition (C + EX). No exercise was conducted during the other low energy availability condition (LEA) and the other control condition (C). Each condition lasted 4 days, and participants underwent each of the four conditions in a randomized order. Participants completed washout periods with ad libitum food intake and exercise for at least 4 days following control conditions and at least 10 days following low energy availability conditions.

Participants

Participants were recruited from our University's campus. Inclusion criteria were (1) male, (2) age: 18–30, (3) ≥3 h of purposeful aerobic exercise per week, (4) body mass index: 19–25 kg · m^{-2}, (5) ≤15% body fat and (6) weight stability (±3 kg) in the past 6 months. Exclusion criteria were (1) smoking, (2) past or present diagnosis of a clinical eating disorder, (3) infectious disease within past 4 weeks, (4) cardiovascular disease or orthopedic impairment that interferes with moderate-to-vigorous exercise, (5) use of medication and (6) diabetes mellitus. The study was approved by the institutional review board of the German Sport University, Cologne. Written informed consent was obtained from all participants prior to study enrolment.

Preliminary assessments

Prior to study start, an experienced nutritionist conducted a diet history interview with each participant, which involved reporting the frequency and quantity of meals, snacks, and beverages consumed during a typical day (Magkos & Yannakoulia, 2003). The purpose of this interview was to assess individual dietary habits and food preferences. Body weight and composition were assessed with a bioimpedance scale (Tanita BC 418 MA, Tanita, Amsterdam, The Netherlands), and peak oxygen uptake (V_{O_2peak}) was assessed using an incremental exercise test on a bicycle ergometer (SRM, Jülich, Germany). Participants started cycling at 1.0 W · kg^{-1} for 5 min; load was increased to 1.5 W · kg^{-1} after 5 min and to 2.0 W · kg^{-1} after another 5 min. After completing 5 min at 2 W · kg^{-1}, load was increased by 20 W every 30 s until volitional exhaustion, which occurred when at least 3 of the following criteria were met: (1) cadence <60 rpm, (2) respiratory exchange ratio ≥1.1, (3) heart rate ≥90%

of age-predicted maximum (220 – age), (4) plateau in oxygen uptake despite increase in load, (5) rating of perceived exertion ≥19 (Borg, 1970). Respiratory data were assessed with a ZAN 600 spirometry system (nSpire Health GmbH, Oberthulba, Germany). Respiratory data collected at 1.0, 1.5 and 2.0 W · kg^{-1} were utilized to determine the load corresponding to 60% V_{O_2peak} and to assess exercise energy expenditure at this intensity. Exercise energy expenditure was calculated using the Weir equation (Weir, 1949).

Energy availability prescriptions

Energy availability was defined as the difference between prescribed energy intake and exercise energy expenditure during supervised exercise (Loucks, 2004). Crude exercise energy expenditure was adjusted for the participants' waking energy expenditure (Loucks et al., 1998), which was calculated as the product of resting energy expenditure, exercise duration and a physical activity level of 1.7. Resting energy expenditure was predicted from FFM (Cunningham, 1991). Because our participants were habitually active but not allowed to conduct any additional exercise, we chose a physical activity level of 1.7, which represents the lower end of moderately active lifestyle spectrum (Westerterp, 2013). Exercise energy expenditure was clamped at 15 kcal · kg^{-1} FFM · day^{-1} in the +EX conditions. Dietary energy intake prescriptions were adjusted individually such that the target energy availability of 15 kcal · kg^{-1} FFM · day^{-1} (LEA and LEA + EX) or 40 kcal · kg^{-1} FFM · day^{-1} (C and C + EX) was reached.

Diet prescriptions

Participants were provided with detailed meal plans for each condition, which specified the type and quantity of food the participant was allowed to eat. Meal plans were developed based on diet habits and food preferences reported during the preliminary interview. Food intake was distributed over 4–6 meals and snacks per day, and for each participant, the distribution of meals and snacks was maintained the same during all conditions. Meal and snack sizes were adjusted such that total daily energy intake matched energy intake prescriptions (Figure 1). Efforts were made to keep macronutrient composition within the recommendations of the German Nutrition Society (50%–55% carbohydrates, 30%–35% fat, 10%–15% protein (DGE, 2012)). Meal plans were explained to each participant in detail so as to clarify food selections and portion sizes. Participants were allowed to consume non-caloric beverages and non-caloric sweeteners ad libitum. All participants were familiar with caloric values and diet logs and were given food scales to weigh consumed food and leftovers. Participants were highly encouraged to report any deviation from their prescribed meal plans. Returned meal plans were analyzed daily using EBIS software (version 7.0, University of Hohenheim, Stuttgart, Germany, 2005). If actual and prescribed energy intake differed by more than 50 kcal on any given day, meal plans for the following days were adjusted such that average energy intake over the whole condition matched energy intake prescriptions.

Exercise expenditure

During both exercise conditions (LEA + EX/C + EX), participants conducted daily supervised exercise on a bicycle ergometer (SRM, Jülich, Germany) at an exercise intensity of 60% V_{O_2peak}. Exercise duration was adjusted individually such that exercise energy expenditure amounted to 15 kcal · kg^{-1} FFM · day^{-1}. Additional exercise and intense physical activity were prohibited. Compliance was monitored using the SenseWear Pro3 armband (Bodymedia, Pittsburgh, USA).

Measurements and assessments

All measurements and assessments were performed in an identical order before (pre) and after completion (post) of each 4-day condition. Participants reported to the lab following an overnight fast of at least 12 h and abstention from exercise for at least 18 h. Participants were instructed to be adequately hydrated. If participants provided a urine with a specific gravity >1.020 g · ml^{-1} (Armstrong, 2007), they were asked to consume 500 ml of tap water and provide another urine before further assessments. Body weight and composition were measured as reported for preliminary assessments. After resting in a supine position for at least 5 min, a blood sample was collected from the antecubital vein.

Hormone and metabolite assay procedures

Serum concentrations of total testosterone, free T3 and insulin were measured on a fully automated system (Advia Centaur, Siemens Healthcare, Eschborn, Germany). Analytical sensitivities were 0.1 ng · ml^{-1} (testosterone), 0.5 pg · ml^{-1} (free T3) and 10 µU · ml^{-1} (insulin). Total precision (intra- and interassay coefficients of variation) were ≤7.6% (testosterone), ≤4.1% (free T3) and ≤7.5% (insulin). IGF-1 was determined on an Immulite 2000 (Siemens Healthcare, Eschborn, Germany; sensitivity: 20 ng · ml^{-1}; precision ≤8.1%). Leptin was assessed using an immunoassay (Mediagnost, Reutlingen, Germany; analytical range: 0.05–5 ng · ml^{-1}; precision ≤7.5%. Ghrelin was determined with a radioimmunoassay (Merck Chemicals, Schwalbach, Germany; sensitivity: 100 pg · ml^{-1}; precision ≤17.8%). Glucose was measured on an Advia (Siemens Healthcare, Eschborn, Germany; sensitivity: 4 mg · dl^{-1}; precision ≤1.9%) and glycerol (Sigma, St. Louis, USA, sensitivity: 0.07 mmol · l^{-1}, precision: ≤11%) and FFA (Wako, Neuss, Germany; sensitivity: 0.07 mmol · l^{-1}; precision: ≤1.5%) were assessed photometrically. Assay results below the limit of detection were adjusted to the lower end of the analytical range.

Statistical analysis

Statistical analyses were performed with R (version 2.14.1). If not stated otherwise, data were reported as mean ± standard error of the mean (SEM), as well as 95% confidence intervals and effect sizes (*d*), when appropriate. With respect to the small sample size, only non-parametric tests were applied. Linear mixed model analysis was used to identify differences in study outcomes and included fixed effect terms for changes from pre to

post measurements (time) and interaction between time and low energy availability (time × low energy availability), between time and exercise (time × exercise), and between time, low energy availability and exercise (time × low energy availability × exercise). To account for repeated measures, the participant identifier was included as a random effect. When time or interaction effects occurred ($P < 0.1$), post-hoc analyses were conducted using paired Wilcoxon rank sum tests. Significance was set at $p < 0.05$, and was adjusted for multiple testing. Sample size was determined based on literature reporting changes in leptin, insulin, T3 and IGF-1 following the reduction in energy availability to 20 kcal · kg^{-1} FFM · day^{-1} in sedentary women (Loucks & Thuma, 2003) and following severe caloric restriction in sedentary men (Dubuc, Phinney, Stern, & Havel, 1998). Based on this data, the expected d was ≥3.2 and a sample size of $n = 6$ was sufficient to detect changes for $d \geq 3.2$ with a power of 0.95.

Results

Demographics

All 6 men who participated in the study completed all four conditions. The participants were 25.2 ± 1.0 years of age, weighed 79.7 ± 3.1 kg and had a body fat percentage of 9.6 ± 1.5% and a V_{O_2peak} of 49.3 ± 2.4 ml · kg^{-1} · min^{-1}.

Compliance with energy prescriptions

In low energy availability conditions, participants consumed on average 15.9 ± 0.2 kcal · kg^{-1} FFM (LEA) and 30.0 ± 0.8 kcal · kg^{-1} FFM (LEA + EX), corresponding to 106 ± 1% (LEA) and 104 ± 2% (LEA + EX) of the prescribed energy intake (Table 1). In control conditions, energy intake was 40.2 ± 0.4 kcal · kg^{-1} FFM (C) and 52.2 ± 2.3 kcal · kg^{-1} FFM (C + EX), corresponding to 101 ± 1% (C) and 97 ± 4% (C + EX) of the prescribed energy intake. Participants attended all supervised exercise sessions as planned and exercised for 91 ± 4 min · day^{-1} at a load of 165 ± 12 W. Actual energy availability in low energy availability conditions was 15.9 ± 0.2 kcal · kg^{-1} FFM (LEA) and 16.0 ± 0.5 kcal · kg^{-1} FFM (LEA + EX), which did not differ from each other ($P = 0.87$). In C conditions, energy availability was 40.2 ± 0.4 kcal · kg^{-1} FFM (C) and 38.3 ± 2.0 kcal · kg^{-1} FFM (C + EX), which also did not differ from each other ($P = 0.42$).

Body weight and body composition

Body weight dropped over the course of both low energy availability conditions, as demonstrated by a time × low energy availability interaction ($P < 0.001$). Weight loss was 2.4 ± 0.3 kg in LEA (95% CI [1.9, 2.9], $d = 1.84$, $P = 0.016$ vs pre) and 1.8 ± 0.4 kg in LEA + EX (95% CI [1.1, 2.5], $d = 1.33$, $P = 0.016$ vs pre). Body weight did not vary as a factor of time or exercise. Participants lost fat mass, as indicated by a trend for a time × low energy availability interaction ($P = 0.09$). Fat mass did not vary as a factor of time or exercise. FFM was not significantly impacted by low energy availability, exercise or time, as there were no significant effects or interaction.

Metabolic hormones

Leptin dropped in both low energy availability conditions, as demonstrated by a time × low energy availability interaction ($P = 0.005$; Table 2). Leptin did not vary over time or with exercise, as there were no other significant effects or interactions for leptin. Post-hoc analysis confirmed that leptin dropped from pre to post by 0.77 ± 0.11 ng · ml^{-1} in LEA (95% CI [0.58, 0.96], $d = 1.66$, $P = 0.018$) and by 0.89 ± 0.13 ng · ml^{-1} in LEA + EX (95% CI [0.68, 1.11], $d = 1.69$, $P = 0.018$). The drop in LEA (Figure 2) was significant when compared to C ($P = 0.02$), as was the drop in LEA + EX when compared to C + EX ($P = 0.01$). The drop in leptin was not significantly different among LEA and LEA + EX ($P = 0.38$).

Insulin dropped in low energy availability conditions, as indicated by a trend for a time × low energy availability interaction ($P = 0.065$). There were no other significant effects or interactions for insulin. Post-hoc analysis confirmed that insulin dropped significantly by 1.50 ± 0.47 µU · ml^{-1} in LEA (95% CI [0.72, 2.28], $d = 1.14$, $P = 0.031$) and by 2.13 ± 0.60 µU · ml^{-1} in LEA + EX (95% CI [1.14, 3.12], $d = 1.21$, $P = 0.031$), and there was a trend indicating that the drop in LEA was smaller when compared to LEA + EX ($P = 0.054$). IGF-1 changed over the course of the study, as indicated by a time effect ($P = 0.042$). For IGF-1, there were also trends for time × low energy availability ($P = 0.078$) and time × exercise ($P = 0.063$) interactions. Post-hoc analyses revealed that IGF-1 tended to increase only in C ($P = 0.09$), but not in any other condition. The change in IGF-1 in C was greater when compared to C + EX (32 ± 8 ng · ml^{-1} vs −6 ± 12 ng · ml^{-1}, $P = 0.03$). For free T3, testosterone and ghrelin, there were no significant effects or interactions (Table 2).

Table 1. Energy intake, exercise expenditure, energy availability and nutrient intake in exercising men (n = 6) during each of the four study conditions.

Condition	Actual Energy Intake (kcal · kg^{-1} FFM · day^{-1})	Prescribed Energy Intake (kcal · kg^{-1} FFM · day^{-1})	Actual to Prescribed Energy Intake (%)	Exercise Energy Expenditure (kcal · kg^{-1} FFM · day^{-1})	Energy Availability (kcal · kg^{-1} FFM · day^{-1})	Carbohydrate Intake (g · kg^{-1} · day^{-1})	Protein Intake (g/kg/day)	Fat Intake (g/kg/day)
LEA	15.9 ± 0.2	15.0 ± 0	106 ± 1	0	15.9 ± 0.2	1.6 ± 0.2	0.8 ± 0.1	0.5 ± 0.1
LEA + EX	30.0 ± 0.8	29.0 ± 0.9	104 ± 2	15	16.0 ± 0.5	3.1 ± 0.3	1.4 ± 0.2	0.9 ± 0.1
C	40.2 ± 0.4	40.0 ± 0	101 ± 1	0	40.2 ± 0.4	4.0 ± 0.2	1.5 ± 0.1	1.4 ± 0.1
C + EX	52.2 ± 2.3	53.9 ± 0.7	97 ± 4	15	38.3 ± 2.0	5.6 ± 0.5	1.8 ± 0.1	1.7 ± 0.1

LEA: low energy availability without exercise, LEA + EX: low energy availability with exercise, C: control without exercise, C + EX: control with exercise.

Table 2. Serum concentrations of metabolic hormones in exercising men (n = 6) before (pre) and after (post) 4 days of low energy availability of 15 kcal · kg^{-1} FFM · day^{-1} attained by caloric restriction without exercise (LEA) or with exercise (LEA + EX) and control conditions (energy availability of 40 kcal · kg^{-1} FFM · day^{-1}) without exercise (C) and with exercise (C + EX).

		Leptin (ng · ml^{-1})		Insulin (μU · ml^{-1})		IGF-1 (ng · ml^{-1})	
	Condition	Pre	Post	Pre	Post	Pre	Post
	LEA	1.49 ± 0.17	0.72 ± 0.15**	4.43 ± 0.60	2.93 ± 0.53*	199 ± 14	203 ± 31
	LEA + EX	1.59 ± 0.28	0.70 ± 0.19*	5.45 ± 0.47	3.32 ± 0.25**	209 ± 19	188 ± 19
	C	1.24 ± 0.20	1.46 ± 0.33	5.07 ± 0.64	4.38 ± 0.50	198 ± 21	230 ± 28
	C + EX	1.40 ± 0.32	1.36 ± 0.26	6.28 ± 1.09	4.57 ± 0.79	207 ± 20	201 ± 14
P-values (Linear Mixed Model)	Time	0.84		0.37		0.042	
	Time × LEA	0.005		0.065		0.078	
	Time × EX	0.64		0.81		0.063	
	Time × LEA × EX	0.81		0.85		0.52	

		Free T3 (pg · ml^{-1})		Testosterone (ng · ml^{-1})		Ghrelin (pg · ml^{-1})	
	Condition	Pre	Post	Pre	Post	Pre	Post
	LEA	3.23 ± 0.07	3.06 ± 0.11	5.40 ± 0.41	5.03 ± 0.85	1192 ± 150	1242 ± 150
	LEA + EX	3.22 ± 0.09	3.03 ± 0.15	5.27 ± 0.46	4.46 ± 0.96	1248 ± 168	1237 ± 205
	C	3.25 ± 0.08	3.16 ± 0.11	5.02 ± 0.26	5.28 ± 0.57	1270 ± 140	1253 ± 170
	C + EX	3.16 ± 0.08	3.36 ± 0.08	4.98 ± 0.47	4.92 ± 0.53	1238 ± 167	1254 ± 201
P-values (Linear Mixed Model)	Time	0.47		0.65		0.89	
	Time × LEA	0.44		0.67		0.93	
	Time × EX	0.14		0.54		0.99	
	Time × LEA × EX	0.23		0.78		0.97	

*, **: Significantly different from pre (P < 0.05, P < 0.01).

Metabolic substrates

There was a reduction in serum glucose in both low energy availability conditions, as demonstrated by a time × low energy availability interaction (P = 0.034; Table 3). Glucose also appeared to be impacted by exercise, as indicated by a trend for a time × exercise (P = 0.050) interaction. There were no other effects or interactions for glucose. Post-hoc analyses confirmed that serum glucose dropped significantly by 6.3 ± 4.2 mg · dl^{-1} in LEA (95% CI [0.67, 13.35], d = 0.78, P = 0.046) and by 9.8 ± 2.4 mg · dl^{-1} in LEA + EX (95% CI [5.79, 13.81], d = 1.35, P = 0.031). Serum glycerol appeared to increase over the course of the study, as indicated by a trend for a time effect (P = 0.059). There were no significant interactions for glycerol. Post-hoc analysis confirmed that glycerol increased in LEA (0.12 ± 0.03 mmol · l^{-1}, 95% CI [0.06, 0.17], d = 1.22, P = 0.029), C (0.06 ± 0.02 mmol · l^{-1}, 95% CI [0.02, 0.10], d = 1.06, P = 0.040) and C + EX (0.02 ± 0.01 mmol · l^{-1}, 95% CI [0.00, 0.033], d = 0.91, P = 0.050). FFA increased during low energy availability conditions, as demonstrated by a time × low energy availability interaction (P = 0.011). There were no other significant effects or interactions for FFA. Posthoc analyses revealed an increase in FFA during LEA (0.22 ± 0.13 mmol · l^{-1}, 95% CI [0.01,0.45], d = 0.84, P = 0.029), LEA + EX (0.19 ± 0.07 mmol · l^{-1}, 95% CI [0.08,0.30], d = 1.08, P = 0.031) and C + EX (0.08 ± 0.02 mmol · l^{-1}, 95% CI [0.04,0.12], d = 1.18, P = 0.018).

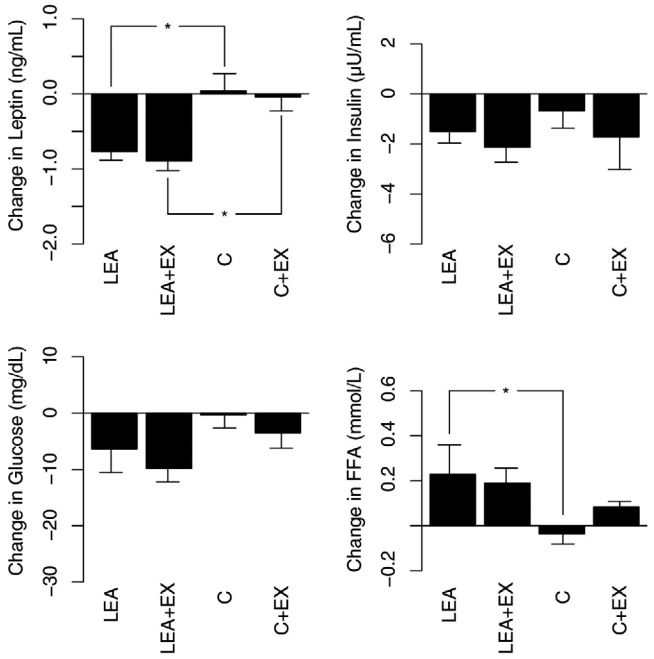

Figure 2. Changes in serum concentrations of leptin, insulin, glucose and FFA in exercising men (n = 6) following 4 days of low energy availability of 15 kcal · kg^{-1} FFM · day^{-1} attained by caloric restriction without exercise (LEA) or with exercise (LEA + EX) and control conditions (energy availability of 40 kcal · kg^{-1} FFM · day^{-1}) without exercise (C) and exercise (C + EX). *Significantly different between conditions (P < 0.05).

Discussion

The purpose of the present study was to determine the metabolic effects of a controlled reduction in energy availability in exercising men and to explore whether these effects differed depending on how low energy availability was attained. Our primary finding was that 4 days of low energy availability (15 kcal · kg^{-1} FFM · day^{-1}) in exercising men was associated with reduced leptin (−53% to −56%), insulin (−34% to −38%) and fasting glucose (−8% to −12%) and increased glycerol (+88% to +167%) and FFA (+70% to +112%) concentrations. Changes in leptin, insulin, glucose and FFA did not differ when low energy availability

Table 3. Serum concentrations of glucose, glycerol and free fatty acids in exercising men (n = 6) before (pre) and after (post) 4 days of low energy availability of 15 kcal · kg^{-1} FFM · day^{-1} attained by caloric restriction without exercise (LEA) or with exercise (LEA + EX) and control conditions (energy availability of 40 kcal · kg^{-1}FFM · day^{-1}) without exercise (C) and exercise (C + EX).

		Glucose (mg · dl^{-1})		Glycerol (mmol · l^{-1})		Free fatty acids (mmol · l^{-1})	
	Condition	Pre	Post	Pre	Post	Pre	Post
	LEA	80.2 ± 3.0	73.8 ± 3.5*	0.07 ± 0.00	0.19 ± 0.03**	0.20 ± 0.06	0.43 ± 0.13*
	LEA + EX	80.6 ± 2.8	70.8 ± 1.6*	0.07 ± 0.01	0.14 ± 0.05	0.27 ± 0.08	0.46 ± 0.10*
	C	80.3 ± 1.8	80.0 ± 2.0	0.07 ± 0.00	0.13 ± 0.02*	0.24 ± 0.04	0.21 ± 0.06
	C + EX	77.8 ± 2.9	74.3 ± 0.9	0.07 ± 0.00	0.09 ± 0.01*	0.17 ± 0.03	0.25 ± 0.03*
P-values (Linear Mixed Model)	Time	0.91		0.059		0.66	
	Time × LEA	0.034		0.11		0.011	
	Time × EX	0.050		0.17		0.59	
	Time × LEA × EX	0.37		0.94		0.90	

*, **: significantly different from pre (P < 0.05, P < 0.01).

was attained through caloric restriction alone or through a combination of caloric restriction and exercise. Contrary to our hypotheses, low energy availability did not affect other metabolic hormones, such as T3, IGF-1, ghrelin and testosterone, in our group of exercising men.

The reduction in leptin that occurred after exercising men were exposed to an energy availability of 15 kcal · kg^{-1} FFM · day^{-1} was in the same magnitude as reported in previous experiments. In sedentary women, Loucks and Thuma (2003) reported reductions in leptin of 54% and 69% after energy availability was reduced to 20 and 10 kcal · kg^{-1} FFM · day^{-1}, respectively (Loucks & Thuma, 2003). No controlled experiments have directly assessed the effects of low energy availability on leptin in men, but a 36% reduction was reported in non-exercising men after their caloric intake was restricted to 840 kcal · day^{-1} for 7 days (Dubuc et al., 1998). Energy availability was not quantified in the latter study, but based on the reported body composition and energy intake data (Dubuc et al., 1998), energy availability was likely in the range of 14 kcal · kg^{-1} FFM · day^{-1}.

It is well documented that the rapid reduction in leptin during acute energy deficiency precedes measurable changes in body composition (Weigle et al., 1997) and involves the loss of normal diurnal variation pattern and decreased 24-h mean concentrations (Chan et al., 2006). Because this reduction in leptin is directly linked to the suppression of key endocrine axes, including the reproductive, growth hormone/IGF-1 and thyroid axes (Chan, Heist, DePaoli, Veldhuis, & Mantzoros, 2003), it has been suggested that it serves as an acute metabolic signal of starvation and energy conservation (Blüher & Mantzoros, 2009). However, despite marked reductions in leptin, we did not observe significant changes in testosterone, IGF-1 and free T3 in either low energy availability condition. To date, no controlled experiments have been published that have established a direct relationship between low energy availability and these metabolic hormones in men, particularly in exercising men. Controlled experiments in sedentary women found that IGF-1 dropped by 31–38% and total T3 dropped by 18% after energy availability was reduced to 10–20 kcal · kg^{-1} FFM (Loucks & Thuma, 2003). In field studies in male soldiers, 25%–50% reductions in IGF-1 and testosterone have been reported during strenuous field training involving energy deprivation (Alemany et al., 2008; Friedl et al., 2000; Kyrolainen et al., 2008; Nindl et al., 2007, 1997). However, these field studies were not conducted under controlled conditions and involved severe energy deficits of 1000–4000 kcal · day^{-1} as opposed to quantifiable measures of energy availability (Alemany et al., 2008; Friedl et al., 2000; Kyrolainen et al., 2008; Nindl et al., 1997, 2007). It is possible that low basal leptin concentrations, which were in agreement with the low body fat percentage (9.6 ± 1.5%), acted to prevent changes in testosterone, IGF-1 and T3. Baseline leptin was already below 2 ng · ml^{-1}, which has been suggested as a threshold for leptin to exert suppressive effects on other endocrine axes (Holtkamp et al., 2003). However, baseline testosterone and IGF-1 concentrations were near the median of age-matched reference ranges (Rosario, 2010; Wang et al., 2008), which suggests that our participants did not show evidence of metabolic and endocrine suppression prior to the study start. It is further possible that the endocrine suppression during low energy availability was blunted by increased circulating concentrations of binding proteins, which were not assessed in this study. Dolan et al. reported that male jockeys, a group known to undergo repeated periods of energy deficiency, demonstrated normal total testosterone but elevated sex hormone-binding globulin concentrations, resulting in reduced testosterone bioavailability when compared to matched controls (Dolan et al., 2012). Future controlled experiments are needed to determine how the magnitude and duration of the energy deficit, basal leptin concentrations and changes in binding proteins modulate the suppression of metabolic hormones during energy deficiency.

In exercising women, the role of metabolic hormone suppression secondary to low energy availability is well established in the etiology of reproductive disorders (De Souza, 2003; Scheid & De Souza, 2010). Reproductive disorders such as reduced sperm motility and quality have also been reported in men participating in energetically expensive sports (De Souza et al., 1994; Lucia et al., 1996), but it remains questionable that the prevalence of reproductive disorders in energy deficient men is as high as that in energy deficient women. Because men have to invest less energy for reproductive purposes than women (Bronson, 1985), it is not surprising that the male reproductive system appears more robust against energy deficiency. Other health-related outcomes of chronic energy deficiency, such as impaired bone health, have also been reported in exercising men (Campion et al., 2010; Dolan et al., 2012). However, the underlying mechanisms, the dose–response relationship with energy availability and the acute vs chronic nature of these effects remain

only poorly understood in this population. Because endocrine data in exercising women match findings from controlled experiments in sedentary women (Loucks, 2004; Loucks et al., 2011), it is unlikely that exercise status protects from endocrine alterations during low energy availability independent of sex.

Despite the absence of the expected changes in IGF-1 and testosterone, low energy availability was associated with alterations in other metabolic pathways. Reductions in insulin (34%–38%) and fasting glucose (8%–12%) were in the same magnitude as previously reported in sedentary women (Loucks & Thuma, 2003). Together with an increased lipolytic activity, as demonstrated by increased serum FFA (+70 to +112%) and glycerol (+88 to +167%), and the concomitant loss of fat mass (0.7–0.8 kg), these findings confirm that reducing energy availability to 15 kcal · kg^{-1} FFM · day^{-1} presented a severe disruption of energy and substrate homeostasis.

Limitations

The present controlled study was designed to assess whether low energy availability has similar metabolic effects in exercising men as those previously reported in sedentary women (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks et al., 1998). We chose to reduce energy availability to 15 kcal · kg^{-1} FFM based on Loucks' experiments, which demonstrated that effects were more pronounced when energy availability was reduced to 10–20 kcal · kg^{-1} FFM · day^{-1} when compared to 30 kcal · kg^{-1} FFM · day^{-1} (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks et al., 1998). In our control condition, energy availability was 40 kcal · kg^{-1} FFM · day^{-1}, which was lower than the 45 kcal · kg^{-1} FFM · day^{-1} that had been operationally defined as balanced energy availability by Loucks and colleagues (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Heath, 1994; Loucks & Thuma, 2003; Loucks et al., 1998). However, mock participants who underwent preliminary testing reported fullness, bloating, constant pressure to eat and weight concerns when consuming the energy intake needed to reach an energy availability of 45 kcal · kg^{-1} FFM · day^{-1}, particularly during the C + EX condition. Thus, we opted to maintain energy availability during control conditions at 40 kcal · kg^{-1} FFM · day^{-1}, which is also supported by field data demonstrating that the habitual energy availability of exercising men is closer to 40 than 45 kcal · kg^{-1} FFM · day^{-1} (Loucks, 2007). Another modification from previous experiments was that energy availability was manipulated by altering the participants' habitual diet as opposed to the use of a clinical dietary product. This was done to allow participants to maximize compliance, but actual energy intake slightly deviated from prescribed energy intake (range: 97%–106%) and actual energy availability during both low energy availability conditions was slightly above the target energy availability (15.9–16.0 kcal · kg^{-1} FFM). This study was conducted in a sample (n = 6) that was slightly smaller than previously used in similar controlled experiments (n = 8–11 [Loucks & Thuma, 2003]). However, previous studies utilized a paired design of two conditions per participant to capture the effects of various levels of energy availability (Loucks & Thuma, 2003), whereas in our study each participant served as their own control during all four study conditions and underwent low energy availability twice. Because the study was adequately powered to detect changes similar to those reported previously (Dubuc et al., 1998; Loucks & Thuma, 2003), we are confident that a larger sample size would not alter the nature of the results.

Conclusion

In exercising men, a short-term reduction of energy availability to 15 kcal · kg^{-1} FFM · day^{-1} is associated with a suppression of leptin and insulin. However, low energy availability did not impact other metabolic hormones, such as IGF-1, free T3 and testosterone, which is contrary to previous controlled experiments in sedentary women. Consequently, exercising men appear to be metabolically more robust against short-term reductions in energy availability when compared to sedentary women. Because these metabolic alterations are linked to long-term health consequences, further research is needed to explore the underlying mechanisms that modulate the differential endocrine and metabolic response to energy deficiency.

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